

# Toxicokinetics of toluene and urinary excretion of hippuric acid after human exposure to $^2\text{H}_8$ -toluene

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## Abstract

Nine male volunteers were exposed to  $^2\text{H}_8$ -toluene (200 mg/m<sup>3</sup> for two hours during a workload of 50 W) via inspiratory air with the aid of a breathing valve and mouthpiece. Labelled toluene was used to differentiate between hippuric acid originating from exposure to toluene and hippuric acid normally excreted in urine. The total uptake of toluene was 2.2 (standard deviation (SD) 0.2) mmol, or 50% of the amount inhaled. Four hours after the end of exposure 1.4 (SD 0.3) mmol or 65% of the total uptake had been excreted in urine as  $^2\text{H}$ -hippuric acid and 20 hours after the end of exposure the cumulative excretion of  $^2\text{H}$ -hippuric acid was 1.8 (SD 0.3) mmol, or 78% of the total uptake. By contrast the cumulative excretion of labelled plus unlabelled hippuric acid exceeded the total uptake of toluene already after four hours. The excretion rate of  $^2\text{H}$ -hippuric acid was highest, about 5  $\mu\text{mol}/\text{min}$ , during exposure and the SD between the subjects was low. The background concentrations of unlabelled hippuric acid in urine were high, however, and there were large differences between subjects. These findings confirm earlier indications that for low exposure, urinary hippuric acid concentration cannot be used for biological monitoring of exposure to toluene.

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Exposure to solvents in the workplace has traditionally been evaluated by measuring the concentration of the solvents in ambient air. Biological monitoring

could present a more accurate estimate of dose, however, and eventually relate better to the health effects of solvents than environmental monitoring of air (1) to compensate for pulmonary ventilation due to the workload, (2) when a solvent is absorbed through the skin rather than or in addition to the respiratory route, (3) when interaction of the chemical with other environmental and workplace chemicals may modify the rate of elimination and thus influence the toxicity of the chemical. For example, the concentration of toluene in blood is increased twofold by simultaneous intake of ethanol.<sup>1</sup> Thus monitoring of environmental concentrations gives misleading information about the internal exposure to toluene.

Toluene is a solvent commonly used in paint, plastics, and printing as well as other chemical industries.<sup>2</sup> There is a well documented dose dependent relation in humans between the concentration of toluene in inhaled air and that in blood.<sup>3</sup> It has also long been known that a strong correlation in humans exists between the toxicity of toluene and the concentration of toluene in inhaled air. The acute neurotoxic effects of toluene increased in severity from mild headache at low doses (50 ppm) to muscular weakness, nausea, and impaired coordination at higher doses (100-200 ppm).<sup>4</sup> Furthermore, reduced perceptual speed, as well as prolonged reaction time, were seen at an exposure level of 300 ppm<sup>5</sup> but not at 80 ppm.<sup>6</sup>

The main metabolic pathway of toluene is the formation of benzyl alcohol, a reaction primarily catalysed by the microsomal cytochrome P-450 system.<sup>7</sup> Further oxidation to benzaldehyde and benzoic acid is catalysed by alcohol and aldehyde dehydrogenases. Thereafter the acid is conjugated with glycine to hippuric acid and around 80% of the absorbed toluene is excreted in the urine as hippuric acid in humans.<sup>8</sup> Besides hippuric acid a small quantity of absorbed toluene (<1%) is oxidised at the aromatic ring and excreted into urine as o- and p-cresol. This has been shown in both animals and humans.<sup>9-13</sup> Between 7 and 14% of the absorbed toluene is excreted unchanged by exhalation in humans.<sup>14</sup>

A biological assay for the evaluation of environmental exposure to toluene is desirable because of the

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high number of exposed workers and because of its toxic effects on the central nervous system.<sup>8 15-17</sup>

Biological monitoring of workers exposed to toluene has been carried out by determining toluene concentrations in blood and expired air.<sup>18-20</sup> The concomitant excretion of hippuric acid and o-cresol in urine of both occupationally<sup>18 21 22</sup> and experimentally<sup>9 23 24</sup> exposed subjects has been evaluated. In several studies it has been shown that the excretion of hippuric acid of exposed workers was significantly correlated with the mean daily environmental concentration.<sup>21 22 25</sup> Hippuric acid is also excreted, however, as a common metabolite of benzoic acid, in people not exposed to toluene. Despite several studies there are still doubts concerning the validity of hippuric acid for biological monitoring, due to the uncertainty regarding the relation between concentrations of toluene in work places and concentrations of urinary hippuric acid.

Most of the benzoic acid is derived from dietary components, whereas a smaller part is excreted as a result of intermediate amino acid metabolism.<sup>26</sup> The dietary components depend mainly on benzoic acid or benzoates that are used as preservatives for several foods—for example, fruit juice, lemonade, bakery products, several dairy products, ketchup and mustard, and some prepared food.<sup>27</sup> In Sweden a maximum of 3 g benzoic acid/kg provisions is admitted. Benzoic acid is rapidly absorbed and thereafter rapidly and completely excreted as hippuric acid in the urine.<sup>26</sup>

In an earlier study of the toxicokinetics of toluene in humans, volunteers were exposed to toluene during standardised exposure to 200 mg/m<sup>3</sup> toluene on three occasions. The excretion of hippuric acid showed large intra-individual variations both before and after exposure.<sup>28</sup> As well as the large intra-individual variations at the three exposure occasions large inter-individual variations were also found.

Because of these large variations an exposure study was designed in which the aim was to follow the excretion of hippuric acid originating from toluene separately from hippuric acid originating from other sources. For this purpose a study with inhalation exposure to <sup>2</sup>H<sub>8</sub>-toluene was conducted.

## Materials and methods

### SUBJECTS

Nine male volunteers, with an average age of 29 (range 18–42) and an average weight of 75 (range 70–90) kg participated in the study. All participants were considered healthy according to a general medical examination preceding the experiment. The subjects were instructed to avoid work with organic solvents and to refrain from alcoholic beverages and drugs for at least two days before the exposure. The study was approved by the regional ethical committee at the Karolinska Institute, Solna, Sweden.

### EXPERIMENTAL DESIGN

The volunteers were exposed to 194 (SD 1) mg/m<sup>3</sup> <sup>2</sup>H<sub>8</sub>-toluene (Aldrich) via inspiratory air with the aid of a valve and mouthpiece. The exposure lasted for two hours during light physical exercise, (workload 50 W) on a bicycle ergometer. The toluene vapour was generated by injection of 46 µl <sup>2</sup>H<sub>8</sub>-toluene with a microlitre syringe into polyester laminated aluminium foil bags filled with 200 l air. After 30 minutes of equilibration the concentration of toluene in the bags was analysed by gas chromatography (3% SE-30 on Chromosorb GAW, 1.5 m, 170°C, nitrogen flow 25 ml/min, flame ionisation detector, Perkin Elmer F11). The error of the method for determination of toluene in the air was +3%.

### TOXICOKINETIC MEASUREMENTS AND ANALYSIS

The total respiratory uptake of toluene during each exposure period was calculated as the difference between the total amount of solvent in inhaled and exhaled air. The exhaled air from each subject was collected in polyester laminated aluminium foil bags for six periods of about six minutes each at regular intervals during the exposures for determination of the expired air volume and the mean pulmonary ventilation was calculated. The concentration of toluene in exhaled air during exposure was analysed as described for samples taken from the respiratory air bags. The relative uptake (R) of toluene for each 20 minute period was calculated from the formula  $R(\%) = (C_{in} - C_{ex})/C_{in} \times 100$ , where  $C_{in}$  and  $C_{ex}$  are the toluene concentrations in inhaled and exhaled air respectively. Total pulmonary uptake (U) was calculated according to the equation  $U = t \times V_E (C_{in} - C_{ex})$ , where  $t$  is the time and  $V_E$  is the pulmonary ventilation corrected to body temperature and pressure and saturated air.

The concentration of toluene in blood was determined before onset of the exposure and at selected intervals during and up to 3 hours after exposure. Capillary blood samples of 200 µl were obtained from a prewarmed finger tip. The blood samples were transferred to gas tight head space bottles that were immediately sealed with teflon lined membranes. After equilibration at 37°C for 30 minutes the head space of the blood samples was analysed by gas chromatography with a head space autosampler technique (0.4% Carbowax A on Carbowax A, 2 m, 160°C, nitrogen flow 30 ml/min, flame ionisation detector, Perkin Elmer F45, Perkin Elmer LC1-100 integrator). The error of the method for determination of toluene in the blood was 4%.

The total amount of urine was collected immediately before onset of exposure and up to 20 hours after exposure. The concentration of labelled and unlabelled hippuric acid in urine was determined by gas chromatography—mass spectrometry (GC-MS) with o-methyl hippuric acid (Aldrich) as inter-

The relative uptake of toluene for each 20 minute period during two hour exposure to  $200 \text{ mg/m}^3$  during physical exercise at a workload of 50 W

Period (min)	Uptake in % of given amount	
	Mean	(SD)
0-20	55.4	(4.4)
21-40	51.8	(7.1)
41-60	48.8	(4.2)
61-80	47.0	(5.0)
81-100	46.1	(5.3)
101-120	46.1	(5.5)
0-120	49.2	(4.8)

nal standard. Urine samples (10 ml) were acidified to pH 1 with concentrated HCl and then run through a Waters  $\text{C}_{18}$ -Sep Pak. The SepPak cartridge was pre-eluted with 2 ml MeOH and 5 ml  $\text{H}_2\text{O}$ . The cartridge was washed with 10 ml 0.01 M HCl and 1 ml benzene. Air was drawn through the cartridge to dryness and the sample eluted with 2.5 ml of  $\text{CHCl}_3$  (1% HAc). The  $\text{CHCl}_3$  fraction was evaporated to dryness, redissolved in 1 ml MeOH, and esterified with 1 ml of  $\text{CHCl}_3/\text{SOCl}_2$  (90:10) for 30 minutes. After evaporation the ester was redissolved in 2 ml of ethyl acetate and analysed by GC-MS.

The mass spectrometric analyses were performed with a Finnigan MAT INCOS 50 GC/MS system equipped with a Varian 3400 gas chromatograph. A capillary column, CP-Sil 8 CB, 24 m  $\times$  0.25 mm internal diameter with 0.13  $\mu\text{m}$  phase was used with helium as carrier gas. One  $\mu\text{l}$  of the sample was injected in split mode at 230°C. The column was operated at 180°C with a carrier gas linear velocity of 27 cm/s. The retention times were: (1) hippuric acid methyl ester 3.4, (2) its deuterated form 3.4, (3) and o-methyl hippuric acid methyl ester 4.1 minutes. Six

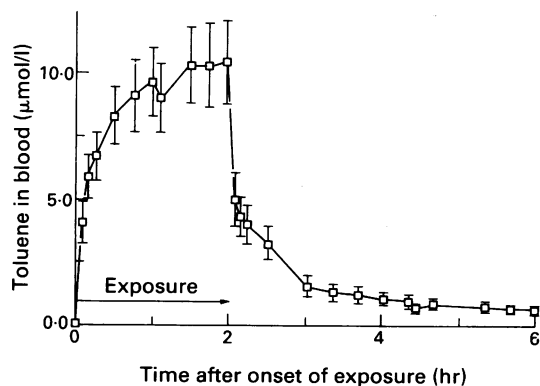


Figure 1 Toluene concentrations in capillary blood during and after exposure for two hours at a workload of 50 W to  $200 \text{ mg/m}^3$  toluene. Mean values and SDs ( $n = 9$ ) are given.

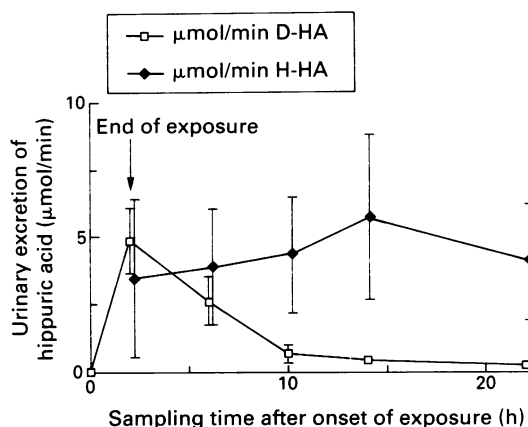


Figure 2 Excretion rate of  $^2\text{H}$ -(D-HA) and unlabelled hippuric acid (H-HA) in urine. Mean values and SDs ( $n = 9$ ) are given.

ions were monitored (selected ion monitoring), with a total scan time of 0.31 s. However only the base peaks were quantified, for (1)  $m/z$  105, (2)  $m/z$  110 and (3)  $m/z$  119. This was performed with the Lab Data System for Chromatography (ELDS) after converting the data files from the INCOS 50 computer. Samples containing 0, 0.5, 1, 2, or 4 g/l of hippuric acid and 1 g/l o-methyl hippuric acid were used to make the calibration curve.

## Results

The total uptake of toluene during two hours of exposure was on average 2.2 (SD 0.2) mmol and represented around 50% of the amount inhaled. During the first 20 minutes of exposure the relative uptake averaged 55% and declined to 46% at the end of exposure. A fairly constant retention was achieved after 60 minutes of exposure (table). During the exposure the concentration of toluene in the blood rose rapidly after the onset of exposure and then levelled off towards the end of exposure (fig 1). The elimination from the blood was considered triphasic during the time studied. The half lives ( $t_{1/2}$ ) of toluene were three minutes for the initial ( $\alpha$ ) phase, 40 minutes for the rapid elimination ( $\beta$ ) phase, and 738 minutes for the slow elimination ( $\gamma$ ) phase.

The excretion rate of  $^2\text{H}$ -hippuric acid was highest, about 5  $\mu\text{mol/min}$ , in the sample taken immediately after the end of exposure. The SDs between subjects were small (fig 2). From the onset of exposure until 11 hours after the exposure the half life was 5.6 hours. The excretion rate of unlabelled hippuric acid was between 3 and 5  $\mu\text{mol/min}$  during the measuring period, and the SDs between subjects were high.

Four hours after the end of exposure 1.4 (SD 0.3) mmol or 65% of the total uptake was excreted in the

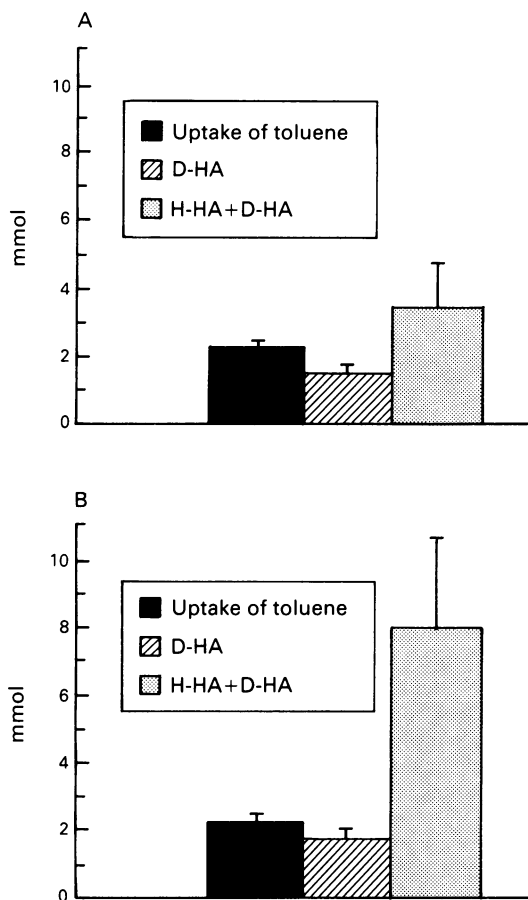


Figure 3 Uptake of toluene and cumulative excretion of  $^2\text{H}$  hippuric acid (D-HA) and the sum of unlabelled and labelled hippuric acid (H-HA + D-HA) A. Four hours and B. 20 hours after the end of exposure. Mean values and SDs ( $n = 9$ ) are given.

urine as  $^2\text{H}$ -hippuric acid and 20 hours after the exposure 1.8 (SD 0.3) mmol or 78% had been excreted (fig 3). The cumulative excretion of both labelled and unlabelled hippuric acid was 3.4 (SD 0.9) mmol already after four hours and thus exceeded the uptake of toluene. After 20 hours the totally excreted amount was 8.0 (SD 2.2) mmol hippuric acid or almost four times higher than the uptake of toluene.

### Discussion

The main quantity of  $^2\text{H}$ -hippuric acid was excreted within four hours after the end of exposure. The highest excretion rate of hippuric acid was found during the exposure. Twenty hours after the exposure about 80% of the dose was excreted as  $^2\text{H}$ -hippuric acid. The excretion of  $^2\text{H}$ -hippuric acid reflected the toluene uptake and the inter-individual

variations were small. The background concentrations of hippuric acid normally found in urine are high, however, and there are large inter-individual variations. Szadkowski *et al* have shown that a meal of food containing benzoic acid caused a threefold and in particular cases a 10-fold increase of hippuric acid excretion.<sup>29</sup> The normal concentration of hippuric acid in the morning urine is 1.2 g/l and after a meal with low intake of benzoic acid 0.75 g/l. Soft drinks may increase the concentration of hippuric acid in urine as much as exposure to 200 mg/m<sup>3</sup> toluene.<sup>27,30</sup> Consequently hippuric acid in urine is questionable as a biological index of exposure to toluene.

These findings confirm the opinion that, for exposures of about 200 mg/m<sup>3</sup> or less, individual hippuric acid concentrations cannot differentiate occupationally exposed subjects from controls.<sup>21,31</sup> A good biological indicator must be capable of evaluating even low exposures, not only in a group of exposed subjects, but also in individual subjects.

o-Cresol has been suggested as a more specific biological test of exposure to toluene.<sup>12,32</sup> Only a very small proportion of the retained toluene is excreted as o-cresol (<1%), however, which raises the question as to whether this metabolite is suitable for biological monitoring at low exposures. It has also been shown that smokers have a three times higher background level of urinary o-cresol excretion compared with non-smokers, probably due to the content of o-cresol in cigarettes.<sup>23</sup>

Biological monitoring by determining toluene concentrations in blood or expired air has been recommended.<sup>18–20</sup> Samples taken during or immediately after termination of exposure only reflect the most recent exposure, however, and not the average exposure during the working day. For samples taken after the end of exposure the sampling time is critical due to the fast decline of the toluene concentration in blood.<sup>14</sup> Blood sampled Monday morning after the exposure reflects the exposure during the preceding week.<sup>33</sup> At low exposures, however, it is difficult to analyse the blood concentrations of toluene.

Earlier studies of urinary hippuric acid concentrations show large variations between subjects.<sup>28,29</sup> In this study the variations of hippuric acid originating from toluene are low between subjects. The variations of hippuric acid from other sources—for example, food intake are high. Even though hippuric acid may be used for biological monitoring of exposure to toluene at workplaces when the exposure is high the conclusion of this study is that when the toluene exposures becomes lower the use of hippuric acid is limited. If hippuric acid excretion in urine is preferred for biological monitoring it is recommended to use diet restrictions to avoid a high intake of benzoic acid during the sampling day and possibly also at least on the day before sampling.

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